VII. *Intersexual forms of* Plebeius argus *L.* (aegon. *Schiff.*),

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Plates V–IX.

In 1916 I published a paper in the Transactions of this Society on *Agriades coridon* Poda, ab. *roystonensis* Pickett. This year through the kindness of a fellow-entomologist I was enabled to visit a colony of *P. argus* in one of the home counties, where a corresponding gynandromorphous or intersexual form occurs.

Geographical Range.

Long series of intersexes of *argus* of this kind existed in the Briggs and Sidney Webb collections from Dover, where it was taken as early as 1864 and as late as 1889. A specimen was recorded from the New Forest (Proc. Ent. Soc. Lond., 1872, p. xliii), and another was taken in the same district by Clark (Entomologist, 1897, xxx, p. 179).

W. Castle-Russell took a considerable number in Surrey in one small locality (Ent. Record, 1917, xxix, p. 211). None could be found amongst the *argus* from neighbouring heaths, but I have seen one taken in 1921 in another part of the county.

Max Wiskott figures one from Oberbayern, in which the wings on the left side with male characters measure 10 mm., and those on the right, which are entirely female, measure 12 mm. (Lepidopteren-Zwitter meiner Sammlung, 1897, Taf. iii, no. 16). Alexander Heyne gives a full description of another taken at Wildenhain near Torgau. The left side, heavily sprinkled with blue and with a black border and whitish fringe, measured 10·5 mm., whereas the right side was brown with a brown fringe, and measured 13 mm. There were orange lunules on both hind-wings and the abdomen was female externally. The left antenna was a little shorter than the right (Rev. Ent. Soc. Namuroise, 1901, pp. 23–24).

Gillmer figures one splashed with blue on all four wings taken by Krodel at Würzburg, May 1901. He says that

the penis protruded, but as the abdomen is female it was probably the ovipositor (Ill. Zeitschr. f. Ent., 1902, vii, p. 211).

Oberthür has figured six with large blue patches, or with the whole of the wings blue on the right side, and with a reduction in size of the wings on the same side. The borders of these wings are very dark and the fringes white. One is from Pléchâtel, and five from Monterfil both in Ille-et-Vilaine, France (Lep. Comp., 1920, xvii, Pl. DX).

Specimens from all these localities agree in having blue scales associated with a reduction in the size of the wing, in most cases confined to the wings of one side. In both Dover and Surrey specimens androconia are present amongst the blue scales. Apart from these two species the same form of intersexuality is found in *Agriades thetis* (*bellargus*).

Oberthür figures two, which are very blue and much smaller on the right side than on the left. One is from Digne, 1907, one from Dompierre-sur-Mer, 1908 (Lep. Comp., 1909, iii, Pl. XIX, figs. 69, 70). He has figured seven more, three with large blue patches on the wings of both sides, four with the blue confined to one side. One is from Auzay, Vendée, and six from Dompierre-sur-Mer, Charente-Inférieure (Lep. Comp., 1920, xvii, Pl. DX).

In these three species intersexes are found year after year in certain restricted localities, but I think it will be discovered that they have a wider range than the present records indicate.

Association with excess of Females and ab. *inaequalis*.

In the case of *A. coridon* there is no doubt that where ab. *roystonensis* is fairly common, as at Royston and near Tring, the number of females greatly exceeds that of the males.

At Alton Barnes, where, so far as I know, only two ab. *roystonensis* have been taken, the sexes appear to be equal in numbers, but a small excess of females would easily pass unnoticed. Castle Russell states that in his Surrey locality for intersexes of *argus* the proportion of females to males was at least 100 to 1.

In the colony of *argus*, which I visited, females did outnumber males, but not to the same extent as in *coridon*

at Royston, although the percentage of intersexes was higher. It is possible that the excess of females and the presence of intersexes may be related phenomena, but observations from other localities are needed.

At Royston *A. coridon* ab. *inaequalis* Tutt is met with every year, and I have taken it with ab. *roystonensis* near Tring. This form has blue streaks or large blue patches on one or more wings, often in situations where no blue is found in any female except ab. *syngrapha*. The blue scales are serrated like those of the female or rounded like those of the male, and beneath and around them are scales so deeply pigmented as to appear black or indigo-blue. There are no coarse hair scales and no androconia, nor is there any reduction in size of the wing. In 1920 two or three were taken with the whole of both wings on one side blue of a deeper colour than ab. *syngrapha* Kefers, and with a very black border. These have no androconia and no hair scales. I regard them as extremely fine examples of ab. *inaequalis*. I am inclined also to think that a single specimen from Royston, which has all four wings entirely of this blue, should be regarded as an example of this condition present on both sides instead of unilateral.

Amongst my *argus* are two which resemble this form of *coridon*. In both of them the wings on the two sides are equal in size. One has three streaks of serrated blue scales and black under scales beneath and around them along the costa of the right fore-wing, the other has a short streak of rounded blue scales with many more black ones near the costa of the right fore-wing; but there are no androconia in either. For this aberration of *argus* with one or more streaks of blue scales, but without androconia or inequality in the size of the wings, and with the coloration of the underside, and the abdomen and genitalia like those of a female, I propose the name ab. *inaequalis* ab. nov.

It seems to be much scarcer than the other intersexual form, just as *A. coridon* ab. *inaequalis* is scarcer than ab. *roystonensis*. Its occurrence in both species in the colonies which produce the intersexes, suggests that these forms may be related to one another. On this point, too, further observation is needed.

In the same place I took a very curious specimen. It is a female with wings symmetrical in size and shape, but on the right side the wings are brown except for a few blue scales at the extreme base, on the left they have a large

extent of blue scaling at the base, that on the hind-wing extending almost to the lunules. The blue scales are serrated and there are no androconia. It seems to me to be a true heterochroic specimen like the one from the New Forest described under the name ab. *duplex* Cockerell. This had the wings on the right side brown, those on the left strongly shaded with blue but not reduced in size. According to Tutt it showed on each half a different form of female colouring and was not a gynandromorph (Bond, Ent. Month. Mag., 1872–73, ix, p. 200; Entomologist, 1889, xxii, p. 6).

Four or five females of *coridon*, showing a different kind of female upperside colouring on the two sides, like these two females of *argus*, have been taken at Royston. It seems to me to be more than a coincidence that all have come from localities where the intersexes are found.

Frequency of Intersexes.

Although the colony is compact and isolated, it is difficult to estimate the percentage of intersexes. On two evenings, when I examined females asleep, I took two out of 230 and one out of 175. Allowing for the fact that some intersexes had been captured previously, at least one per cent. of the total number of females must be intersexes. During the daytime I took them at about the same rate as on these two evenings, and I think this estimate is fairly correct. I am sure the percentage of intersexes of *argus* in this colony is much higher than that of *coridon* ab. *roystonensis* at Royston or near Tring.

Habits.

The flight and general behaviour of the intersexes resemble those of females. Twice I saw one being pursued by a male, and it quivered its wings and took up the attitude of a normal female. I am told that a number have been found paired, and my dissections show that this happens frequently. Three of my specimens laid a few eggs and they were laid singly in the usual way. The eggs were fertile and one larva has died after eating a hole in the shell.

External Characters.

All my 58 specimens have androconia mixed with the blue scales even where these are few in number. The blue

scales are nearly all rounded like those of a male instead of being serrated, and they are usually grouped in streaks and patches rather than peppered over the wing as they are in *coridon*. In a very large number blue scales are found in positions where no blue occurs even in the bluest *P. argus* ab. *masseyi* Tutt.

Blue scales often completely replace the orange of the lunules in the fore- or hind-wing and extend beyond the lunules. In some a few blue scales are found amongst the orange ones, or a thin stripe of blue runs right through a lunule. This unusual extension of blue outwards was noted in 46 of my series and probably is present in others. In intersexes with much blue near the margin the margin is blackened like that of a male and the fringe is white, or there are white patches in it. This is clearly visible in the figures. Thus the following male characters may be present :—

(1) androconia,
(2) rounded blue scales often situated in places where no blue is present in the bluest females,
(3) black margin,
(4) white fringe.

The underside is nearly always entirely female in coloration. The reduction in size of the blue-scaled wings is less than in *coridon* unless the blue scaling is very extensive. One specimen, however, has a very small left hind-wing without any blue scales, and a left fore-wing with a blue streak and only a small indentation of the margin adjoining it. In two specimens the antenna on the side with male characters is smaller than the other. Fifty-four specimens have blue on one side only; nineteen of them have blue on one fore-wing only, three on one hind-wing only, and thirty-two on both wings. Four have blue on the wings on both sides, two of them on all four wings, one on both hind-wings and the right fore-wing and one on both fore-wings. There is a higher proportion of specimens with a bilateral distribution of blue scales and androconia than in *coridon*. I have seen three taken in 1918 with all four wings nearly blue all over, so that the appearance of the upperside was much more that of a male than a female. I have one taken at Dover in 1889 exactly like a male on the upperside except for small patches of orange scales representing part of lunules 1, 2 and 4 on the left

hindwing and 2 and 4 on the right. Even the underside is like that of a male, but the abdomen has no long hairs and has female genitalia (Pl. VI, fig. 3).

Anatomy of Internal and External Genitalia.

I dissected 26 intersexes and 29 normal females as controls, but most of these came from the same colony and there may be more than the average amount of variation amongst them. In every case the external genitalia, ovipositor and rods were normal and no trace of any male structure was found. The prop and rein with the chitinous apparatus for the attachment of the muscles which extrude them were normal. In these respects they agree with intersexes of *coridon*.

The bursa copulatrix with its caput was always present, but showed remarkable variations in size and shape. The ductus bursae or seminis was invariably present and normal. The spermatheca was present in all cases and had the two dilated portions found in normal females, but the size and shape of these varied a good deal. The cement glands showed abnormalities in two examples. In one, intersex no. 7, of which the abnormal ovaries are figured, the proximal dilated part of each gland was very narrow and short, that on the right being half and that on the left a third the usual size, the distal tubular part being normal. In another the dilated portion was rather narrow and tapering. In the majority the ovaries were large and contained well-developed eggs. Many showed evidence of having laid eggs and had eggs in the common oviducts and vagina. The eggs in these situations lay with their long axes vertical instead of horizontal as in the egg-tubes. Only three had abnormal ovaries.

In intersex no. 7, a specimen with a moderate amount of blue scaling on the right fore-wing, there was a small ill-shaped egg with sculptured shell in the vagina. In the left ovary the outer tube had a single well-developed ovum with normal shell, but the younger ova above were not nearly so far developed as in normal tubes. In the second tube all the ova were very immature. Such a backward development is not found even in newly emerged females, which always have at least two ova in each egg-tube with green contents and sculptured shell. In the third tube the first ovum was aborted and represented only by a few granules. The

fourth tube had one well-developed ovum, and all the rest were very immature and apparently abnormal. In the right ovary in the outermost tube there was not even a dilatation where the first ovum should have been, and the ova actually present were all very backward in development. In the second tube the first ovum was aborted and the rest very immature, in the third tube the first ovum was still more obviously aborted. In the fourth tube the first ovum in the fresh condition was represented by an oval mass of granular material much bigger than a full-sized normal ovum. This owing to lack of density has shrunk in alcohol, and the figure gives a poor idea of its original bulk. In this ovary there was not a single ovum which appeared to be normal (Pl. IX, fig. 5).

In intersex no. 25, a specimen with only a little blue on the left side, there were three ova in the vagina, of which the lowest and highest were normal. The middle one was very small, with dense homogeneous green contents like those of a normal ovum, and with a small crinkled shell with deficient sculpturing. There was a normal ovum in each common oviduct. In the left ovary the lowest eggs were normal, but three are depicted end on owing to twisting of the egg-tube in preparation. They lay with long axis vertical and were moving down the oviduct. The smaller ova were very immature, and not all at corresponding stages of development at corresponding points in each tube, as they are in a normal ovary. In the right ovary in one tube the lowest ovum was small and misshapen, but had dense green contents and a shell small but sculptured. The ovum above it was normal, as were the lowest ova in the next two tubes. The lowest ovum in the fourth tube was absent (Pl. IX, fig. 6). The third abnormal specimen had only three instead of four egg-tubes in each ovary, but the ova were normal. This specimen laid two eggs in captivity. The figure of it gives a good idea of the size of a normal egg-tube for comparison with the small abnormal tubes of the other two (Pl. IX, fig. 4).

The ova with dense green contents indicating maturity are unshaded, the less mature with granular yellowish contents are shaded in all the diagrams.

Amongst the intersexes of *coridon* a specimen with similar ovaries, each with only three egg-tubes, was discovered. In a hundred normal Lycaenids of various species every ovary had four egg-tubes.

The normal bursa copulatrix in *A. coridon* consists of a tube of uniform width ending in an oval dilatation, the caput bursae. It arises from a point slightly below the apex of a rounded chitinous prominence, broader at the base than the apex and flattened in its antero-posterior diameter. This is supplied with muscles for extruding the prop and rein of Chapman. These organs are continuous with the tubular part of the bursa, and at the point of junction the ductus bursae arises. The ductus in *argus* is double the length of the tubular part of the bursa. The first half is narrow and the second half which opens into the vagina is much wider.

In *argus* bursae like those of *coridon* were found in 25 out of 29 normal females, *i. e.* females with no male characters, but only in 8 intersexes out of 26. There were minor variations in the length and width of the tubular part, and in the size of the caput, in specimens which I have regarded as normal.

In the intersexes abnormalities of the tubular part were common. The proximal part of the tube arising from the basal prominence was frequently more or less dilated, and the dilatation extended a variable distance up towards the caput. Then it became suddenly narrower, forming a tube thinner than the tubular part of a normal bursa. This thin part was of considerable length in some and in others formed a mere constriction between the dilated part and the caput. In one there were two constrictions in the tubular part, and in another one constriction in the tube and a second in the caput itself. In one case the bursa was lying very much twisted and the tubular part, narrow at its origin, became gradually wider and then suddenly constricted just below the caput, which was quite transparent. In one specimen, which dried after having been in alcohol, the dilatation became full of air and was seen to have very thin walls. Several appeared to be like this, but in others a narrow inner tube ran down the tubular part of the bursa and the dilated part almost to its point of origin. In a normal bursa this inner tube can be traced from near the point of origin up the tube into the caput, where it expands into an oval termination.

In *Rumicia phlaeas* there is always a short, broad dilatation of the bursa before the narrow tubular part commences, and perhaps this corresponds to the proximal dilated portion of the bursal tube in these abnormal *argus*.

In two specimens the whole bursa was very small and narrow, both the tubular part and the caput, and in another the tube was long and wide and the caput short and broad (Plate I, fig. 8). One had a constriction of the caput as well as a dilatation of the proximal part of the tubular portion.

My figures, which are drawn to scale, show these abnormalities and also the great variation in the total length of the tubular part and in the size of the caput. A shrunken wrinkled caput is probably normal, and merely indicates loss of contents and contraction of the muscle within. The caput has a single layer of cubical epithelium covering the thick chitin, on the inner side of which numerous tracheae run. Inside this is a thick layer of circular muscle fibres.

Four intersexes had a transparent caput and were probably virgins. In many it contained some opaque yellow material like that found in worn normal females. These had probably been impregnated some days before. In several it was full of dense white and brown contents such as I have found in *argus* and *coridon* taken *in cop.* These had been impregnated recently. Amongst the controls two had bursae with a shrivelled, square-ended caput and a narrow tubular part dilating about half-way down into a wider tube. Two others with abnormal dilatations are figured (Plate IX, figs. 1 and 2).

The bursae were examined in most cases immediately after death, and rough sketches made, which agreed closely with the more careful drawings made after preservation in alcohol.

Comparison of the Genitalia of Intersexes of *argus* and *coridon*.

The intersexes of *argus* agree with similar specimens of *coridon* in having normal female external genitalia. Amongst the *coridon* one with small cement glands and one with a bursa constricted between tube and caput were found like some of the abnormal *argus*. In both species a specimen was met with which had only three egg-tubes in each ovary instead of four. But no *coridon* was found with abnormal ova in the ovaries like the two *argus*, and no argus was found with complete absence of bursa and ductus bursae, or with a bifid termination to the spermatheca, which were abnormalities found in *coridon*.

General Remarks.

I think these insects must be regarded as intersexes and not as true gynandromorphs or sex mosaics. Morgan has proved conclusively that the latter are due nearly always to the loss of an X-chromosome occurring usually at the first division of the fertilised ovum. True gynandromorphs of both *coridon* and *argus* are known, and prove that the secondary sexual characters in these species are carried in the X-chromosome. If these insects were due to an abnormality of the X-chromosome one would expect to find male structures in their internal and external genitalia and in their gonads.

True intersexes in Lepidoptera are found in various primary and secondary hybrids, of which a list is given in my paper in the Journal of Genetics. All of them replace females. Nuttall and Keilin have found intersexes replacing females in lice, chiefly in hybrids or crosses between head and body lice.

Goldschmidt has studied intersexuality in *Lymantria dispar* in crosses between strong and weak races. In the first cross between a male of a strong race and a female of a weak race he obtained 50% males and 50% intersexes or 100% males. Half the latter proved to be transformed males and had only one X-chromosome like females instead of two like normal males. Male intersexes, individuals with two X-chromosomes but with some female secondary sexual characters, appeared in later generations. He considers that the intersexuality is due to a difference of potency in the factors for maleness and femaleness in different races of this species.

Sturtevant has found large numbers of sterile intersexes in a race of *Drosophila simulans*. He has given convincing proof that they are females modified by a recessive autosomal mutant gene, which causes them to show male structures. Their sex glands are absent or very minute. He has proved that the male parts have two X-chromosomes in their cells like the female parts. It is interesting that this gene or factor is carried not in a sex chromosome but in an autosomal one. He has shown also that in true gynandromorphs of *D. simulans* the cells of the male parts contain only one X-chromosome.

Bridges has found another kind of sterile intersex in *Drosophila melanogaster*. In these the gonads are rudi-

mentary ovaries, ovotestes or an ovary and a testis. He has shown by cytological examination that these intersexes all possess the second and third (autosomal) chromosomes in triplicate and the X-chromosome in duplicate, and that some have the fourth chromosome in triplicate and some in duplicate, and some have a Y-chromosome and others are without it. He draws the conclusion that in this species the sex is due to a balance between the X and the autosomal chromosomes, the fourth chromosome having genes with a disproportionately large influence in producing male characters. Of the four kinds of intersex differing in their chromosome complex, two were recognisable in their structural characters. Those with a triploid fourth chromosome were mainly male, those with a diploid fourth chromosome were mainly female in structure. Using X for the X or sex chromosome and A for each set of autosomal chromosomes, individuals with 2X : 2A, 3X : 3A and ? 1X : 1A were all females, although in normal *Drosophila* those with 1X are males, those with 2X : 3A were intersexes and those with 3X : 2A or 1X : 3A were sterile females and sterile males respectively. The original brood from which he obtained his intersexes produced 96 females, 9 males and 80 intersexes. Ten per cent. of the females were structurally unlike the rest and these produced more intersexes.

It is evident that intersexes in insects may be produced in different ways and that every case requires special investigation. The intersexes of *Agriades* and *Plebeius* differ in certain respects from any others, notably in the unilateral distribution of male characters in the great majority of them. They appear to have most in common with Bridges' *Drosophila*. In these there was a great excess of females and intersexes over males, and the intersexes themselves were of more than one kind. The Lycaenid intersexes occur in places where females are in great excess over males, and it is not unlikely that the intersexes are of two kinds, a commoner one with reduction of the size of the wings and with blue scales and androconia scattered over them, and a rarer one with no alteration in size of wings, with streaks or large patches of blue scaling but without androconia.

The abnormality of the chromosomes cannot be identical with that of Bridges' *Drosophila*, because in Lepidoptera the female is the sex with only one X-chromosome and

therefore heterozygous for sex, whereas in *Drosophila* the reverse is the case. In Lepidoptera the male determining factor is carried by the X-chromosome and the female determining factor appears to be carried by the autosomal chromosomes. Nevertheless, it seems to me likely that these Lycaenid intersexes possess an unusual number of chromosomes and the number is probably an uneven one. This would explain the restriction of male characters to one side, and might also explain the origin of the females with a different pattern on the two sides.

Summary.

Two kinds of female intersex are found in *A. coridon* and *P. argus*, and in each the male characters are much more often unilateral than bilateral.

The first kind is much commoner than the second and has blue scales, androconia, and other male characters. The wings with male characters are reduced in size. The bluest bilateral ones look more like males than females. The gonads and genitalia are female.

The second kind has no androconia and no male character except blue scales. The blue scales form streaks or large patches, or may in *coridon* cover the whole of both wings on one side except the margins. Very rarely streaks of blue are found on the wings of both sides. A specimen of *coridon* from Royston referred to ab. *syngrapha* is more likely to be a completely blue intersex.

Intersexes of the first kind may be fertile. Those of the second kind have not been tested. There is a great excess of females where these intersexes are found, and females with a different upperside pattern on the two sides have been met with in the same localities. Intersexes in both species occur wild year after year in the same localities, and the geographical range is wide.

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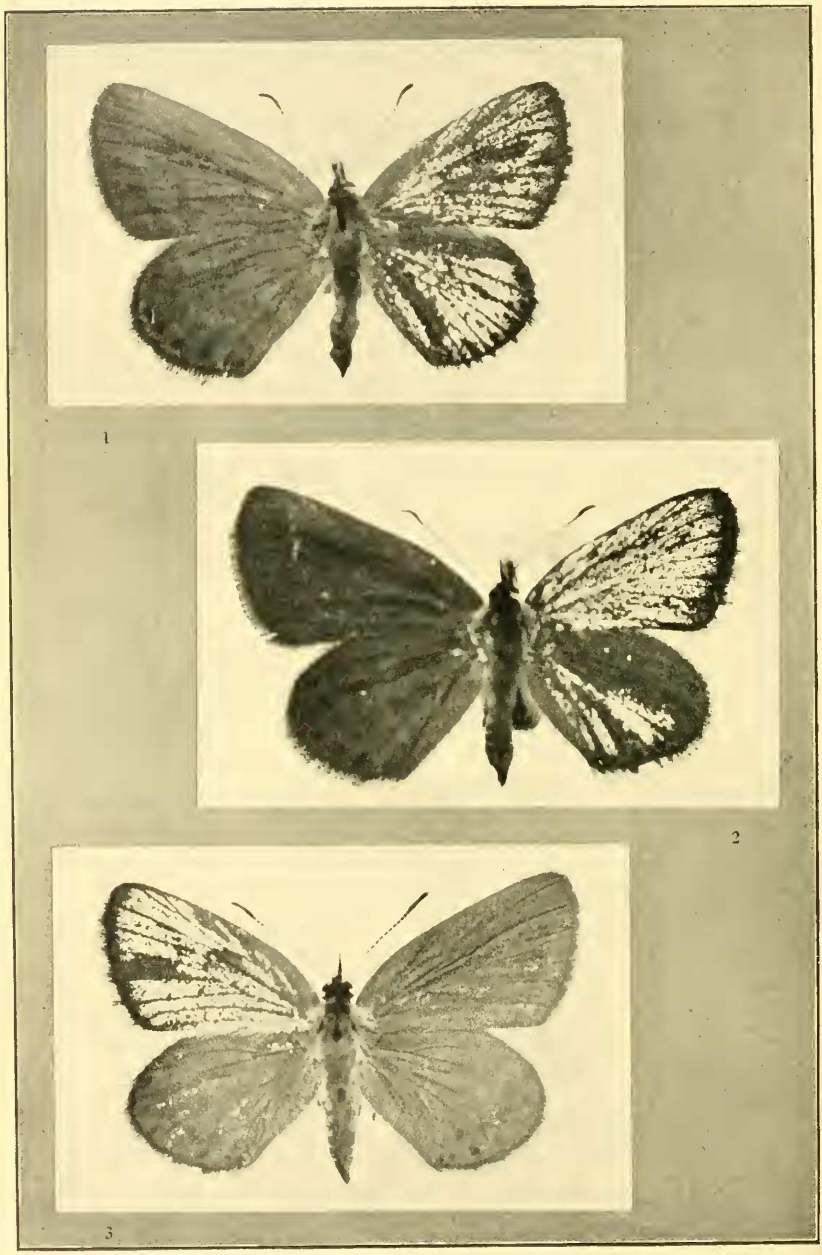

INTERSEXES OF PLEBEIUS ARGUS